International Journal of Applied Pharmaceutics

ISSN- 0975-7058

Original Article

THE POTENTIAL EFFECT OF APORPHINE ALKALOIDS FROM *NELUMBO NUCIFERA* GAERTN. AS ANTI-BREAST CANCER BASED ON NETWORK PHARMACOLOGY AND MOLECULAR DOCKING

ADRIAN[1], MUHAMMAD FAUZAN LUBIS[2*], RONY ABDI SYAHPUTRA[3], RIRIN ASTYKA[2], SUMAIYAH SUMAIYAH[4], MUHAMMAD ANDIKA YUDHA HARAHAP[5], ZAHRATUL AINI[3]

[1]Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia. [2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. [3]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. [4]Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. [5]Faculty of Animal Sciences, Universitas Brawijaya, Malang, Indonesia

[*]Corresponding author: Muhammad Fauzan Lubis; [*]Email: fauzan.lubis@usu.ac.id

Received: 19 Aug 2023, Revised and Accepted: 20 Oct 2023

ABSTRACT

Objective: To demonstrate the efficacy and benefits of aporphine alkaloids from *Nelumbo nucifera* Gaertn. as anti-breast cancer agents.

Methods: In this study, a combination of network pharmacology and molecular docking was used to investigate the pharmacological actions and underlying mechanisms of action of nuciferine, nor-nuciferine, and roemerine against breast cancer.

Results: Fifty-five potential targets of compounds against breast cancer were identified. The Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB), and Protein Kinase C Epsilon (PRKCE) were identified as the top five targets of compounds against breast cancer. Molecular docking demonstrated that these compounds could bind spontaneously to the screened top 4 targeted proteins.

Conclusion: The present study demonstrates that these compounds have pharmacological effects against breast cancer *via* a multi-target and multi-pathway manner.

Keywords: Nuciferine, Nornuciferine, Roemerine, Breast cancer, Network pharmacology, Molecular docking

DOI: https://dx.doi.org/10.22159/ijap.2024v16i1.49171 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Breast cancer is a prevalent form of malignancy among women worldwide and has emerged as a primary cause of mortality [1]. Breast cancer constitutes 24.2% of the total 8.6 million newly diagnosed cases among females and accounts for 15.0% of all 4.2 million cancer-related mortalities [2]. Over the past few decades, there has been a noticeable increase in the incidence of breast cancer in both developed and developing nations [3]. This trend is particularly evident in younger age groups [4]. Breast cancer has emerged as a significant health concern in Indonesia, surpassing other forms of cancer, with a prevalence of 16.6% [5]. This poses a serious threat to the well-being and survival of women in the country [6].

Currently, contemporary medical practice predominantly employs surgical interventions, radiotherapy, chemotherapy, and hormone therapy as primary modalities for addressing breast cancer in patients [7]. Notably, the treatment objectives for breast cancer patients include preserving quality of life and extending life expectancy [8]. However, it is pertinent to mention that breast surgery is not typically considered a conventional treatment modality in most cases [9]. While chemotherapy has demonstrated efficacy in short-term treatment, prolonged treatment is often associated with unfavorable reactions and drug resistance, which can have a detrimental impact on patients' quality of life and physical and mental well-being [10]. Therefore, scholars are enthusiastic about discovering viable alternative treatments [11].

Recently, there has been a growing focus on utilizing traditional medicine as a means of treating cancer [12]. This is primarily due to its noteworthy clinical effectiveness, minimal adverse effects, and ability to enhance overall quality of life [13]. Traditional Indonesian Medicine known as an anticancer agent is Seroja or *Nelumbo nucifera* Gaertn. Seroja, a member of the Nelumbonaceae family, is an aquatic plant that possesses medicinal properties in nearly all its constituent parts [14]. Many studies have reported the anticancer activity of various parts of Seroja in anti-pancreatic cancer, anti-liver cancer, anti-cervix cancer, anti-colon cancer, and anti-breast cancer [15, 16]. This activity was demonstrated by the secondary metabolites of Seroja. The main compound found in all parts of Seroja is an aporphine alkaloid [17]. Pharmacological studies have shown that aporphine alkaloids possess diverse activities, such as metabolic regulation, antioxidation, anti-proliferation, anti-vascularization, apoptosis induction, and cell cycle arrest inhibition [15, 18]. The cellular targets of this phenomenon remain unknown.

The present investigation utilized network pharmacology analysis to anticipate the potential therapeutic targets and signaling pathways of aporphine alkaloids for the treatment of breast cancer. Hopkins established network pharmacology in 2007, which has been demonstrated as a potent approach for investigating the intricate and comprehensive activation mechanisms of natural products [19]. This text depicts the complex interplay between genes, proteins, and metabolites in diseases and drugs. This is achieved by integrating various multidisciplinary concepts such as biochemistry, bioinformatics, and systematic biology [20]. These concepts align with the multifaceted characteristics of natural products [21]. The integration of network pharmacological prediction and molecular docking verification has emerged as a crucial approach to elucidate the primary molecular targets and underlying mechanisms of action of herbal medicines before conducting *in vitro* and *in vivo* experiments [22]. The aim of this study was to elucidate the molecular mechanisms underlying the anti-breast cancer activity of aporphine. alkaloids from Seroja using network pharmacology and molecular docking techniques.

MATERIALS AND METHODS

Compound database

The identification of aporphine alkaloid compounds derived from Seroja was accomplished through a comprehensive review of the relevant literature. Three aporphine alkaloid compounds, nuciferine,

nor-nuciferine, and roemerine, were acquired. The database was queried using the simplified molecular input line entry (SMILE) notation of the compounds sourced from the PubChem database, namely "nuciferine, nor-nuciferine, and roemerine," as the search terms.

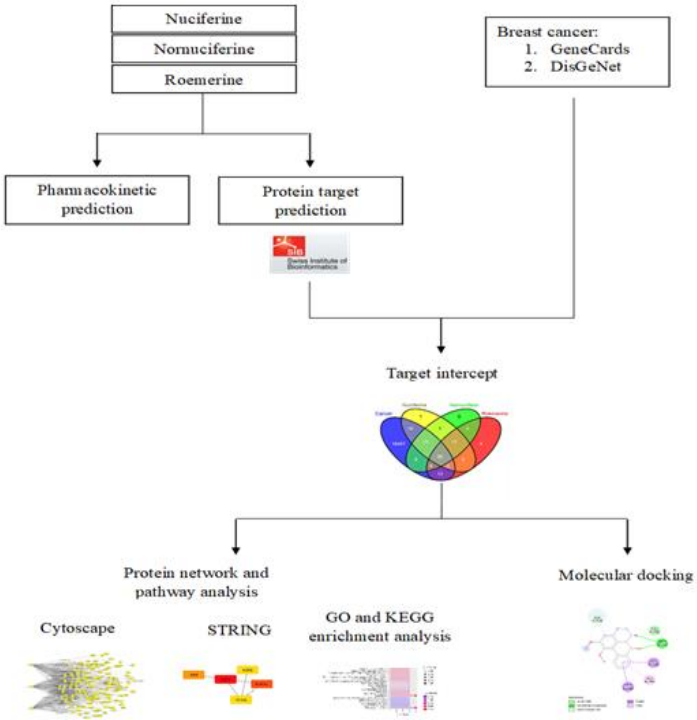

Fig. 1: The framework of this study

Pharmacokinetic prediction

The molecular weight, log P, H-bond acceptor, H-bond donors, rotatable bond, % intestinal absorption, Blood-Brain Barrier (BBB), toxicity, hepatotoxicity, and maximum tolerated dose (mg/kg/day) pharmacokinetic parameters of nuciferine, nornuciferine, and roemerine were acquired using the pkCSM tools, which can be accessed via https://biosig.unimelb.edu.au/pkcsm/[23].

Compound-target identification

The canonical SMILE of the compounds was uploaded to the Swiss Target Prediction database (http://www.swisstargetprediction.ch/)[24]. The compound-target network was visualized using Cytoscape (3.9.0)[25].

Breast cancer target identification

Breast cancer targets were retrieved by searching the GeneCards database (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/) were integrated using VENNY (https://bioinfogp.cnb.csis.es/tools/venny_old/index.html), and the intersecting targets were presented in a Venn diagram [26].

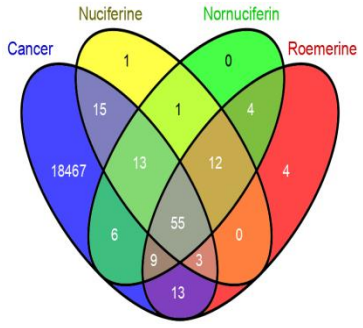

Fig. 2: Venn diagram representing the overlapping of breast cancer targets and compound

Construction of protein-PPI network

The Protein-protein interaction (PPI) network was constructed by uploading the genes to the STRING v_11.0 database (https://string-db.org/) [27]. The settings for building the PPI network were established in accordance with the "Homo sapiens" model, and the confidence level of the interaction between the targets was set at 0.9 [28]. The network nodes represent proteins, and the edges reflect the protein-protein interactions. For core target protein identification, the Cytoscape data were sorted using these parameters, including degree, closeness centrality, betweenness centrality, and clustering coefficient [26].

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were analyzed using the ShinyGO database (http://bioinformatics.sdstate.edu/go/) with "homo sapiens" as a species with a False Discovery Rate (FDR) cutoff of 0.05 [29, 30].

Molecular docking

The possible interactions between the compounds nuciferine, nor-nuciferine, and roemerine with the targets, including Epidermal Growth Factor Receptor (EGFR, PDB ID 5hg8), Mitogen-Activated Protein Kinase 8 (MAPK8, PDB ID 3elj), Janus Kinase 2 (JAK2, PDB ID 5aep), and Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB, PDB ID 4kik) were modelled. Each protein was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/pages/policies). The interaction between each compound and its target was predicted using PyRx Virtual Screening Tools (version 0.8). All interactions between the target proteins and compounds were constructed using PyMOL 2.5 in the PDBQT format file. Finally, 2-dimensional visualization of the interaction between compounds and target proteins was investigated using Discovery Studio Visualizer 2021 [31, 32].

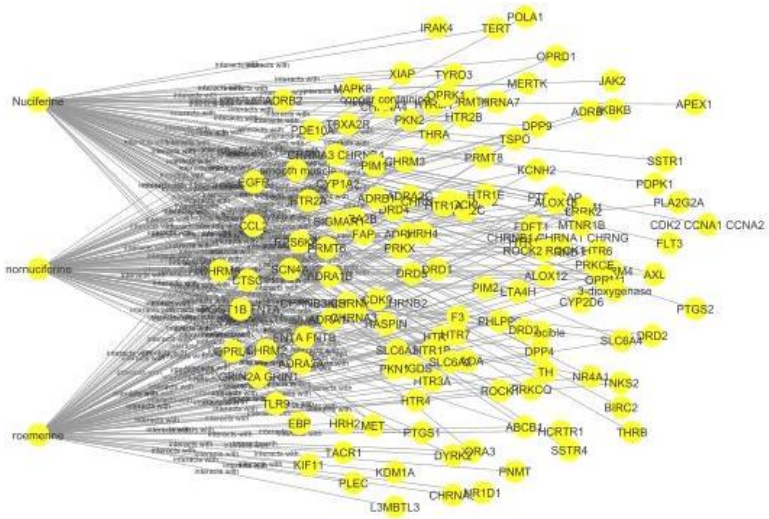

Fig. 3: The network of compound and proteins target constructed using Cytoscape 3.9.0

RESULTS

Pharmacokinetic parameters of compounds

The pharmacokinetic parameters of nuciferine, nornuciferine, and roemerine were determined using the pkCSM tools. This investigation was used to screen for interactions of the compounds with the human body. Ten parameters were used, as listed in table 1. These results indicate that nuciferine, nor-nuciferine, and roemerine are suitable compounds for consumption.

Identification of drug targets in breast cancer

The experimental procedure is illustrated in fig. 1. Targets in breast cancer were searched in the GeneCards Human database and DisGeNet using "breast cancer" as keywords. After removing duplicates among these databases, 18467 breast cancer targets were identified based on the relevance score (fig. 2). The targets of each aporphine alkaloid were obtained from Swiss Target Prediction. The identified predicted targets included 100 targets of nuciferine, nor-nuciferine, and roemerine. The 55 intersecting targets of aporphine alkaloids and breast cancer are shown in a Venn diagram (fig. 2) and listed in table 1. Furthermore, a compound-target network plot was constructed using Cytoscape 3.9.0 (fig. 3).

Analysis of target protein-protein interaction (PPI) network

The 55 intersecting targets were imported into the STRING database, and the potential relationships among them were investigated. A PPI network with a confidence score of 0.9 was constructed (fig. 4.). STRING database analysis revealed that the average node degree, defined as the average number of interactions of a protein in a network, was 3.93, and the local clustering coefficient, defined as the wellness of the connected nodes in a network, was 0.651. The interactions between the targets comprised 30 nodes and 59 edges, with each edge representing an association between nodes. Cytoscape analysis revealed one main cluster associated with the PPI network (fig. 4). According to the scores of degrees, closeness centrality, betweenness centrality, and clustering centrality, signal transducer and activator of Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB), and Protein Kinase C Epsilon (PRKCE) were identified as the top five intersecting targets of aporphine alkaloid and breast cancer interaction (fig. 4).

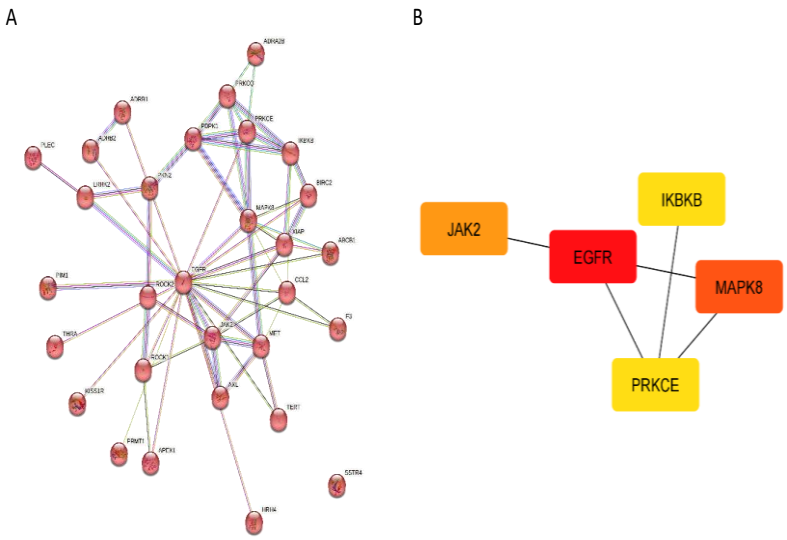

Fig. 4: Protein-protein interaction (PPI) analysis. (A) Protein-protein interaction network of nuciferine, nornuciferine, roemerine, and breast cancer targets obtained from STRING v_11.5 and imported into Cytoscape. (B) The top 5 targets in the PPI network as ranked using the cytoHubba plug in network analyzer. The higher degree value is represented by colors ranging from red to yellow

Table 1: Pharmacokinetic parameters of nuciferine, nornuciferine, and roemerine

No	Parameters	Compounds		
		Nuciferine	Nornuciferine	Roemerine
1	Molecular weight	295.382	281.355	279.339
2	Log P	3.4559	3.1137	3.1674
3	H-bond acceptor	3	3	3
4	H-bond donors	0	1	0
5	Rotatable bond	2	2	0
6	% Intestinal absorption	96.604	93.767	96.771
7	BBB (log BB)	0.418	0.743	0.321
8	Ames toxicity	No	No	No
9	Hepatotoxicity	No	No	No
10	Max. tolerated dose (mg/kg/day)	-0.23	-0.575	-0.456

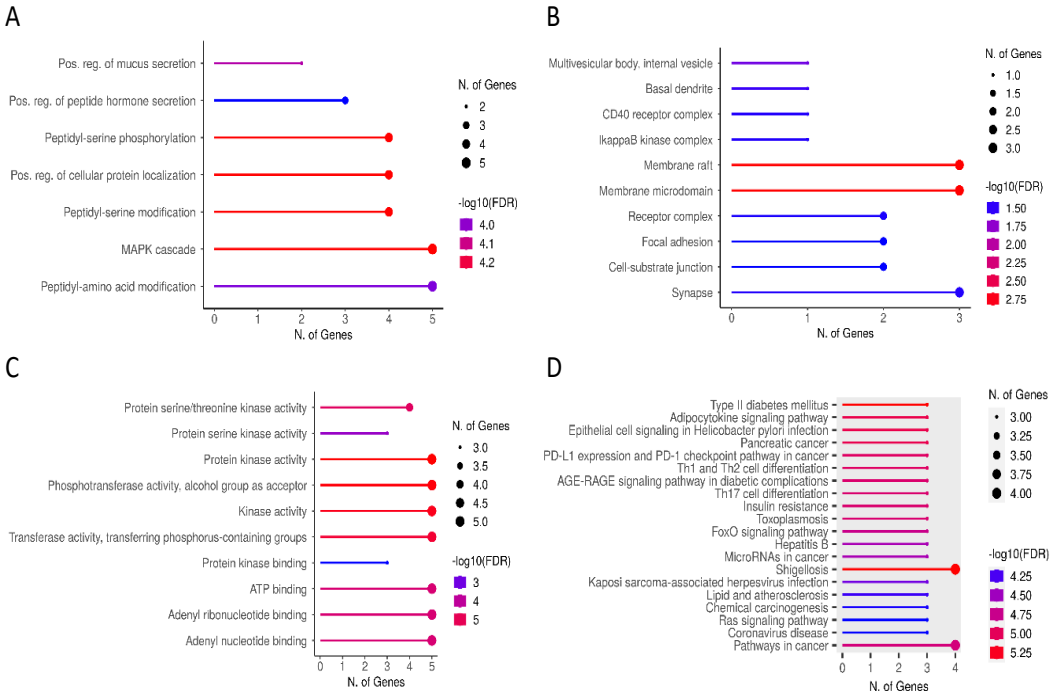

Fig. 5: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The biological processes (A), cellular component (B), molecular function (C), and KEGG terms (D) distributed in the ordinate and the degree of enrichment were analyzed. The size of the dots represents the gene count. The blue-to-red represents the low-to-high value of the enrichment

Gene ontology (GO) and Kyoto Encyclopedia of genes and genomes (KEGG) pathway analysis

GO enrichment analysis was conducted to analyze the impact of the targets in breast cancer. The classification of GO was based on three criteria: biological processes, GO molecular function, and GO subcellular localization (fig. 5). First, data with the candidate target and KEGG pathway analysis were interpreted using the ShinyGO database. The top seven biological processes of aporphine alkaloids were identified by false discovery rate (FDR) and sorted by fold enrichment, including positive regulation of mucus secretion, positive regulation of peptide hormone secretion, peptidyl-serine phosphorylation, positive regulation of cellular protein localization, peptidyl-serine modification, Mitogen-Activated Protein Kinase (MAPK) cascade, and peptidyl-amino acid modification (fig. 5). Molecular function was mainly enriched in protein serine/threonine kinase activity, protein serine kinase activity, protein kinase activity, phosphotransferase activity, alcohol group as acceptor, kinase activity, transferase activity transferring phosphorus-containing groups, protein kinase binding, adenosine 5'-triphosphate (ATP) binding, adenyl ribonucleotide binding, and adenyl nucleotide binding (fig. 5). Finally, subcellular localization includes multivesicular body internal vesicles, basal dendrites, Cluster of Differentiation 40 (CD40) receptor complex, IkappaB kinase complex, membrane raft, membrane microdomain, receptor complex, focal adhesion, cell-substrate junction, and synapses (fig. 5). Furthermore, the KEGG pathway analysis suggested that the predicted targets were components of the pathways involved in oncogenesis, particularly in the Programmed Death Ligand 1 (PD-L1) expression and Programmed Death 1 (PD-1) checkpoint pathway in cancer, Forkhead Box O (FoxO) signaling pathway, microRNAs in cancer, Ras signaling pathways, and pathways in cancer (fig. 5).

Compounds-target interaction analysis by molecular docking

Among the 55 targets of nuciferine, nornuciferine, and roemerine in breast cancer, four potential targets, including EGFR, MAPK8, JAK2, and IKBKB, were investigated for possible interactions with nuciferine, nornuciferine, and roemerine. The aporphine alkaloids were molecularly docked using PyRx Virtual Screening Tool, and their interactions with the highest affinity for each target are presented in fig. 6, and table 2. The major binding interactions included hydrogen bonding and van der Waals interactions. The binding energy score during docking indicates the affinity of a component for the target protein. Here, all the binding energy scores analyzed were less than 0, suggesting high-affinity interactions among the targets and the aporphine alkaloids (nuciferine, nornuciferine, and roemerine) (table 2) [33, 34].

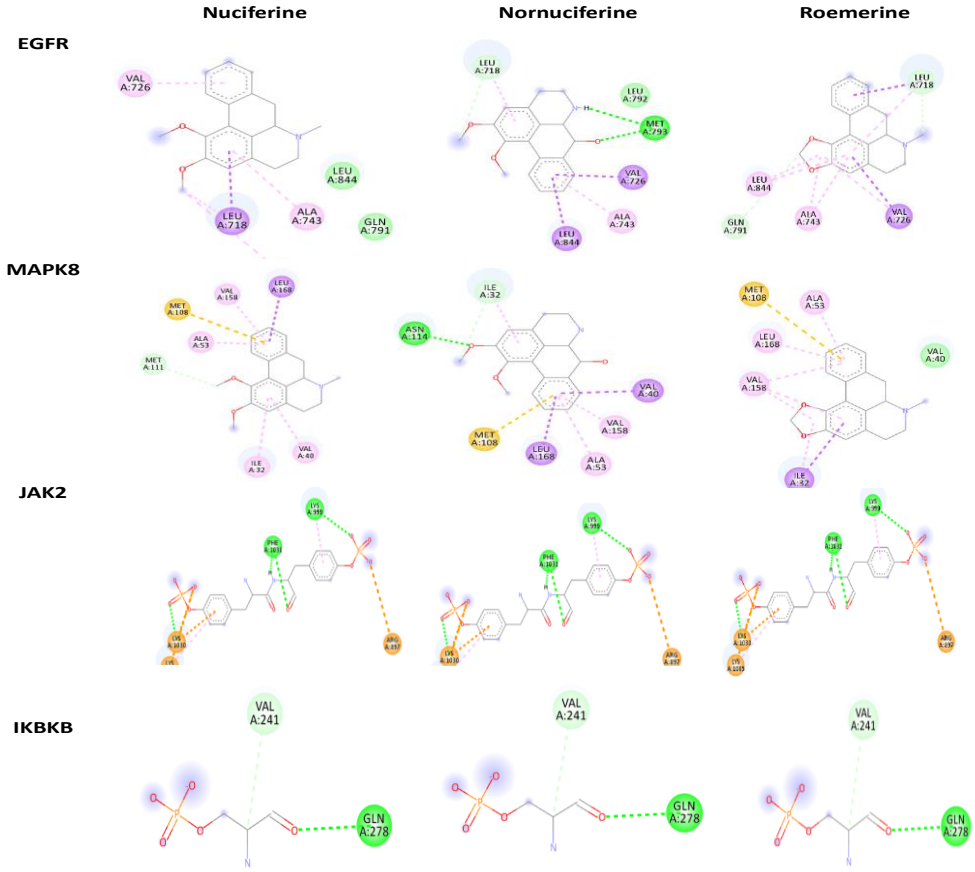

Fig. 6: Molecular docking between nuciferine, nornuciferine, and roemerine with EGFR, MAPK8, JAK2, and IKBKB. Hydrogen bonds were displayed in green and Van der Waals interactions were displayed in light green

Table 2: The molecular docking results of nuciferine, nornuciferine, and roemerine

Compounds	Molecular docking							
	Binding energy (kcal/mol)				Chemicals bond			
	EGFR	MAPK8	JAK2	IKBKB	EGFR	MAPK8	JAK2	IKBKB
Nuciferine	-7.3	-9.2	-9.2	-7.5	Van der waals (LEU844 dan GLN791)	Hydrogen (MET111)	Hydrogen (PHE1031 and LYS999)	Hydrogen (VAL241 and GLN278)
Nornuciferine	-7.7	-9.1	-9.3	-6.9	Hydrogen (MET793) and Van der waals (LEU718 and LEU792)	Hydrogen (ASN114 and ILE32)	Hydrogen (PHE1031 and LYS999)	Hydrogen (VAL241 and GLN278)
Roemerine	-8.6	-10.0	-9.9	-8.0	Hydrogen (LEU718 and GLN791)	Van der waals (VAL40)	Hydrogen (PHE1031 and LYS999)	Hydrogen (VAL241 and GLN278)

DISCUSSION

The utilization of network pharmacology-based evaluations facilitates comprehensive investigations of the interactions between potential drug candidates and diverse network factors [35]. The integration of multidisciplinary concepts from biological systems and polypharmacological models has led to the emergence of potent tools for drug target identification [36]. In addition to identifying novel drugs and their corresponding targets, this tool facilitates the investigation of potential target regions and repurposing of established drugs for a variety of ailments [37]. The present study delineates the molecular mechanism underlying the interaction between nuciferine, nornuciferine, and roemerine, which are aporphine alkaloids derived from Seroja.

A previous study demonstrated that aporphine alkaloids, namely nuciferine, nornuciferine, and roemerine, exhibit antiproliferative effects in AGS and DU-145 cells [15]. Nuciferine inhibited the growth of MDA-MB-231 and MCF-7 cells in the context of breast cancer by inducing apoptosis and inhibiting proliferation through cell cycle arrest [38]. However, the molecular mechanisms underlying this effect remain unknown. Elucidating the molecular mechanisms of aporphine alkaloids can enhance their anti-breast cancer activity. This study identified potential targets of compounds that overlap with breast cancer targets, such as Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), and Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB). Based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, these targets are important for the advancement of breast cancer [39-42].

EGFR expression has been detected in a range–14-91% of individuals diagnosed with breast cancer and has been associated with an unfavorable prognosis [43]. Activation of EGFR triggers the activation of various pathways, such as RAS/RAF/ERK, PI3K/AKT, and STAT3 [44]. These pathways have been found to play a crucial role in regulating the growth and progression of cancer [45, 46]. The MAPK8 pathway is crucial in breast cancer and is similar to the EGFR protein [47]. MAPK8, also called c-Jun NH-2 terminal kinase-1 (JNK1), belongs to the MAPK gene family [48]. The pathway above has been documented to be active in the process of apoptosis in cases of breast cancer [49]. JAK2 is a member of the MAPK family

and has been detected in breast cancer [50]. Proteins involved in the JAK-STAT pathway have been shown to undergo modifications in breast cancer. These alterations can occur through various mechanisms, such as downregulation of phosphortyrosine-specific phosphatases, elevation of the JAK/STAT activating ligand IL-6, activation of other upstream oncogenic pathways such as ErbB1 or PI3K/mTOR, and downregulation of STAT3 negative regulators such as suppressor of cytokine signaling 3 [51-53]. The most recent protein identified as a target of nuciferine, nor-nuciferine, and roemerine was IKBKB. IKBKB has been identified as a constituent of NF-KB signaling, and its heightened activity has been observed in breast cancer [54]. In certain instances, upregulation of IKBKB has been observed to result in a reduction in the responsiveness of cancer cells to chemotherapy [55].

The present study employed molecular docking analysis to investigate potential targets for their interactions with nuciferine, nor-nuciferine, and roemerine. The results of our study support our initial hypothesis, as the compounds in question exhibited strong binding affinity for the designated targets. It is probable that these compounds effectively hindered the functions of EGFR, MAPK8, JAK2, and IKBKB, which are known to promote tumor growth [56-58]. Furthermore, there is a dearth of information pertaining to aporphine alkaloids, namely, nuciferine, nornuciferine, and roemerine, in various databases. Thus, the present findings represent an inaugural report on this subject. Further clarification is required regarding the interactions between drugs and their respective targets, and it is imperative to validate the pharmacokinetic profile, safety, and efficacy of drugs. This study has provided scientific insights into this compound, indicating its potential for use in anti-cancer drug research and development for clinical purposes.

CONCLUSION

The present study described the potential activity of aporphine alkaloids from Seroja as anti-breast cancer agents through network pharmacology and molecular docking tests, which illustrated that these compounds are suitable for development as oral drugs because the parameters were correct, including toxicity tests. Taken together, using a combination of network pharmacology and molecular docking, we demonstrated that nuciferine, nornuciferine, and roe-merine as aporphine alkaloids can suppress breast cancer via modulation of Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), an Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB). This study confirmed that roemerine has the lowest binding energy compared with nuciferine and nornuciferine against EGFR, MAPK8, JAK2, and IKBKB. We recommend continuous efforts to demonstrate the anti-breast cancer activity of roemerine through *in vitro* and *in vivo* tests.

ACKNOWLEDGMENT

We appreciate the help of the laboratory staff of the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Muhammad Fauzan Lubis: Conceptualization, Supervision, Methodology, Data curation, Validation, Formal analysis, Investigation, Funding acquisition, Writing–original draft. Adrian: Data curation, Validation, Formal analysis, Writing–original draft. Rony Abdi Syahputra: Data curation, Validation, Formal analysis, Writing–original draft. Ririn Astyka: Resources, Data curation, Conceptualization, Methodology, Formal analysis, Writing–original draft. Sumaiyah Sumaiyah: Project administration, Software, Formal analysis, Writing–original draft. Muhammad Andika Yudha Harahap: Data curation, Validation, Formal analysis, Writing–original draft. Zahratul Aini: Project administration, Software, Formal analysis, Writing–original draft.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019 Jan 22;321(3):288-300. doi: 10.1001/jama.2018.19323, PMID 30667505.
2. Lukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment strategies-an updated review. Cancers (Basel). 2021 Aug 25;13(17):4287. doi: 10.3390/cancers13174287, PMID 34503097, PMCID PMC8428369.
3. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY. Risk factors and preventions of breast cancer. Int J Biol Sci. 2017 Nov 1;13(11):1387-97. doi: 10.7150/ijbs.21635, PMID 29209143, PMCID PMC5715522.
4. Almansour NM. Triple-negative breast cancer: a brief review about epidemiology, risk factors, signaling pathways, treatment and role of artificial intelligence. Front Mol Biosci. 2022 Jan 25;9:836417. doi: 10.3389/fmolb.2022.836417, PMID 35145999, PMCID PMC8824427.
5. Anwar SL, Raharjo CA, Herviastuti R, Dwianingsih EK, Setyoheriyanto D, Avanti WS. Pathological profiles and clinical management challenges of breast cancer emerging in young women in Indonesia: a hospital-based study. BMC Womens Health. 2019 Feb 6;19(1):28. doi: 10.1186/s12905-019-0724-3, PMID 30728000, PMCID PMC6364389.
6. Widiana IK, Irawan H. Clinical and subtypes of breast cancer in Indonesia. Asian Pac J Cancer Care. 2020;5(4):281-5. doi: 10.31557/apjcc.2020.5.4.281-285.
7. Tong CWS, Wu M, Cho WCS, To KKW. Recent advances in the treatment of breast cancer. Front Oncol. 2018 Jun 14;8:227. doi: 10.3389/fonc.2018.00227, PMID 29963498, PMCID PMC6010518.
8. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM. Clinical diagnosis and management of breast cancer. J Nucl Med. 2016 Feb;57Suppl 1:9S-16S. doi: 10.2967/jnumed.115.157834, PMID 26834110.
9. Abrahams HJG, Gielissen MFM, Schmits IC, Verhagen CAHHVM, Rovers MM, Knoop H. Risk factors, prevalence, and course of severe fatigue after breast cancer treatment: a meta-analysis involving 12 327 breast cancer survivors. Ann Oncol. 2016 Jun;27(6):965-74. doi: 10.1093/annonc/mdw099, PMID 26940687.
10. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res. 2017 Oct 2;50(1):33. doi: 10.1186/s40659-017-0140-9, PMID 28969709, PMCID PMC5625777.
11. Zuo S, Wang Z, An X, Wang J, Zheng X, Shao D. Self-assembly engineering nano drugs composed of paclitaxel and curcumin for the combined treatment of triple-negative breast cancer. Front Bioeng Biotechnol. 2021 Aug 24;9:747637. doi: 10.3389/fbioe.2021.747637, PMID 34504835, PMCID PMC8421550.
12. Panth N, Paudel KR, Karki R. Phytochemical profile and biological activity of Juglans regia. J Integr Med. 2016 Sep;14(5):359-73. doi: 10.1016/S2095-4964(16)60274-1, PMID 27641607.
13. Luo H, Vong CT, Chen H, Gao Y, Lyu P, Qiu L. Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine. Chin Med. 2019 Nov 6;14:48. doi: 10.1186/s13020-019-0270-9, PMID 31719837, PMCID PMC6836491.
14. Hu P, Ge X, Gao M, Wang X, Zhang Y, Li Y. Nelumbo nucifera Gaertn: an updated review of the antitumor activity and mechanisms of alkaloids. Pharmacological Research-Modern Chinese Medicine. 2022;5. doi: 10.1016/j.prmcm.2022.100167.
15. Liu CM, Kao CL, Wu HM, Li WJ, Huang CT, Li HT. Antioxidant and anticancer aporphine alkaloids from the leaves of Nelumbo nucifera gaertn. cv. rosa-plena. Molecules. 2014 Nov 3;19(11):17829-38. doi: 10.3390/molecules191117829, PMID 25372397, PMCID PMC6271390.
16. Zhao X, Feng X, Wang C, Peng D, Zhu K, Song JL. Anticancer activity of *Nelumbo nucifera* stamen extract in human colon cancer HCT-116 cells *in vitro*. Oncol Lett. 2017 Mar;13(3):1470-8. doi: 10.3892/ol.2016.5547, PMID 28454279, PMCID PMC5403397.

17. Yang ZD, Zhang X, Du J, Ma ZJ, Guo F, Li S. An aporphine alkaloid from Nelumbo nucifera as an acetylcholinesterase inhibitor and the primary investigation for structure-activity correlations. Nat Prod Res. 2012;26(5):387-92. doi: 10.1080/14786419.2010.487188, PMID 21732870.
18. Jin Q, Yang D, Dai Z, Khan A, Wang B, Wei X. Antitumor aporphine alkaloids from Thalictrum wangii. Fitoterapia. 2018 Jul;128:204-12. doi: 10.1016/j.fitote.2018.05.012, PMID 29772303.
19. Wang Y, Zhang Y, Wang Y, Shu X, Lu C, Shao S. Using network pharmacology and molecular docking to explore the mechanism of Shan Ci Gu (*Cremastra appendiculata*) against non-small cell lung cancer. Front Chem. 2021 Jun 9;9:682862. doi: 10.3389/fchem.2021.682862, PMID 34178945, PMCID PMC8220148.
20. Wang K, Wang Y, Yan J, Hou C, Zhong X, Zhao Y. Network pharmacology and molecular docking integrated strategy to the screening of active components and mechanisms of stephaniae tetrandrae radix on breast cancer. Processes. 2022;10(11):2340. doi: 10.3390/pr10112340.
21. Zhang X, Shen T, Zhou X, Tang X, Gao R, Xu L. Network pharmacology based virtual screening of active constituents of *Prunella vulgaris* L. and the molecular mechanism against breast cancer. Sci Rep. 2020;10(1):15730. doi: 10.1038/s41598-020-72797-8, PMID 32978480.
22. Li ZH, Yu D, Huang NN, Wu JK, Du XW, Wang XJ. Immunoregulatory mechanism studies of ginseng leaves on lung cancer based on network pharmacology and molecular docking. Sci Rep. 2021;11(1):18201. doi: 10.1038/s41598-021-97115-8, PMID 34521875.
23. Syahputra HD, Masfria M, Hasibuan PAZ, Iksen I. *In silico* docking studies of phytosterol compounds selected from *Ficus religiosa* as potential chemopreventive agent. Rasayan J Chem. 2022;15(2):1080-4. doi: 10.31788/RJC.2022.1526801.
24. Daina A, Michielin O, Zoete V. Swiss target prediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019 Jul 2;47(W1):W357-64. doi: 10.1093/nar/gkz382, PMID 31106366, PMCID PMC6602486.
25. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov;13(11):2498-504. doi: 10.1101/gr.1239303, PMID 14597658, PMCID PMC403769.
26. Iksen I, Witayateeraporn W, Wirojwongchai T, Suraphan C, Pornputtapong N, Singharajkomron N. Identifying molecular targets of aspiletrein-derived steroidal saponins in lung cancer using network pharmacology and molecular docking-based assessments. Sci Rep. 2023;13(1):1545. doi: 10.1038/s41598-023-28821-8, PMID 36707691.
27. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta Cepas J. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019 Jan 8;47(D1):D607-13. doi: 10.1093/nar/gky1131, PMID 30476243, PMCID PMC6323986.
28. Von Mering C, Jensen LJ, Snel B, Hooper SD, Krupp M, Foglierini M. STRING: known and predicted protein-protein associations, integrated and transferred across organisms. Nucleic Acids Res. 2005 Jan 1;33:D433-7. doi: 10.1093/nar/gki005, PMID 15608232, PMCID PMC539959.
29. Yi Y, Fang Y, Wu K, Liu Y, Zhang W. Comprehensive gene and pathway analysis of cervical cancer progression. Oncol Lett. 2020 Apr;19(4):3316-32. doi: 10.3892/ol.2020.11439, PMID 32256826, PMCID PMC7074609.
30. Chen J, Zhang D, Shen B, Zhang X, Xu B, Xu X. Enantioseparation of D, L-α-amine acid on crown ester chiral stationary phases. Fenxi Huaxue Chin J Anal Chem. 2006;34(11):1535-40.
31. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010 May;24(5):417-22. doi: 10.1007/s10822-010-9352-6, PMID 20401516, PMCID PMC2881210.
32. Dallakyan S, Olson A. Participation in global governance: coordinating' the voices of those most affected by food insecurity. Glob Food Secur Gov. 2015;1263:1-11. doi: 10.1007/978-1-4939-2269-7.
33. Then R. Dihydropteroate synthase. X Pharm Compr. Pharmacol Ref. 2007:1-7. doi: 10.1016/B978-008055232-3.60524-0.
34. Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci Adv. 2016 Mar 25;2(3):e1501240. doi: 10.1126/sciadv.1501240, PMID 27051863, PMCID PMC4820369.
35. Jin J, Chen B, Zhan X, Zhou Z, Liu H, Dong Y. Network pharmacology and molecular docking study on the mechanism of colorectal cancer treatment using Xiao-Chai-Hu-Tang. Plos One. 2021 Jun 14;16(6):e0252508. doi: 10.1371/journal.pone.0252508, PMID 34125845, PMCID PMC8202922.
36. Bai H, Wang R, Li Y, Liang X, Zhang J, Sun N. Network pharmacology analysis, molecular docking, and *in vitro* verification reveal the action mechanism of *Prunella vulgaris* L. in treating breast cancer. Evid Based Complement Alternat Med. 2022 Aug 10;2022:5481563. doi: 10.1155/2022/5481563, PMID 35990843, PMCID PMC9385303.
37. Song Y, Sakharkar MK, Yang J. Probing the mechanism of action (MOA) of *Solanum nigrum* linn against breast cancer using network pharmacology and molecular docking. SN Appl Sci. 2023;5(5). doi: 10.1007/s42452-023-05356-1.
38. Kang EJ, Lee SK, Park KK, Son SH, Kim KR, Chung WY. Liensinine and nuciferine, bioactive components of *Nelumbo nucifera*, inhibit the growth of breast cancer cells and breast cancer-associated bone loss. Evid Based Complement Alternat Med. 2017;2017:1583185. doi: 10.1155/2017/1583185, PMID 29333179, PMCID PMC5733186.
39. Tang X, Jin L, Cao P, Cao K, Huang C, Luo Y. MicroRNA-16 sensitizes breast cancer cells to paclitaxel through suppression of IKBKB expression. Oncotarget. 2016 Apr 26;7(17):23668-83. doi: 10.18632/oncotarget.8056, PMID 26993770, PMCID PMC5029655.
40. Balko JM, Schwarz LJ, Luo N, Estrada MV, Giltnane JM, Davila Gonzalez D. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. Sci Transl Med. 2016 Apr 13;8(334):334ra53. doi: 10.1126/scitranslmed.aad3001. Erratum in: Sci Transl Med. 2019 Jan 23;11(476). PMID 27075627, PMCID PMC5256931.
41. Gong L, Tang H, Luo Z, Sun X, Tan X, Xie L. Tamoxifen induces fatty liver disease in breast cancer through the MAPK8/FoxO pathway. Clin Transl Med. 2020 Jan;10(1):137-50. doi: 10.1002/ctm2.5, PMID 32508033, PMCID PMC7240857.
42. Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. Breast Cancer Res Treat. 2012 Nov;136(2):331-45. doi: 10.1007/s10549-012-2289-9. PMID 23073759, PMCID PMC3832208.
43. Maennling AE, Tur MK, Niebert M, Klockenbring T, Zeppernick F, Gattenlöhner S. Molecular targeting therapy against EGFR family in breast cancer: progress and future potentials. Cancers (Basel). 2019 Nov 20;11(12):1826. doi: 10.3390/cancers11121826, PMID 31756933, PMCID PMC6966464.
44. Ali R, Wendt MK. The paradoxical functions of EGFR during breast cancer progression. Signal Transduct Target Ther. 2017;2:16042. doi: 10.1038/sigtrans.2016.42, PMID 28435746.
45. Ma JH, Qin L, Li X. Role of STAT3 signaling pathway in breast cancer. Cell Commun Signal. 2020 Feb 28;18(1):33. doi: 10.1186/s12964-020-0527-z, PMID 32111215, PMCID PMC7048131.
46. Rocca A, Braga L, Volpe MC, Maiocchi S, Generali D. The predictive and prognostic role of RAS-RAF-MEK-ERK pathway alterations in breast cancer: revision of the literature and comparison with the analysis of cancer genomic datasets. Cancers (Basel). 2022 Oct 28;14(21):5306. doi: 10.3390/cancers14215306, PMID 36358725, PMCID PMC9653766.
47. Xu P, Zhang G, Hou S, Sha LG. MAPK8 mediates resistance to temozolomide and apoptosis of glioblastoma cells through MAPK signaling pathway. Biomed Pharmacother. 2018 Oct;106:1419-27. doi: 10.1016/j.biopha.2018.06.084, PMID 30119215.

48. Semba T, Wang X, Xie X, Cohen EN, Reuben JM, Dalby KN. Identification of the JNK-active triple-negative breast cancer cluster associated with an immunosuppressive tumor microenvironment. J Natl Cancer Inst. 2022 Jan 11;114(1):97-108. doi: 10.1093/jnci/djab128, PMID 34250544, PMCID PMC8755499.
49. Zhai CL, Tang GM, Qian G, Han BJ, Hu HL, Wang SJ. miR-190 protects cardiomyocytes from apoptosis induced by H_2O_2 through targeting MAPK8 and regulating MAPK8/ERK signal pathway. Int J Clin Exp Pathol. 2018 Apr 1;11(4):2183-92. PMID 31938330, PMCID PMC6958226.
50. Liu Q, Ai B, Kong X, Wang X, Qi Y, Wang Z. JAK2 expression is correlated with the molecular and clinical features of breast cancer as a favorable prognostic factor. Int Immunopharmacol. 2021 Jan;90:107186. doi: 10.1016/j.intimp.2020.107186, PMID 33290964.
51. Zhu N, Zhang J, Du Y, Qin X, Miao R, Nan J. Loss of ZIP facilitates JAK2-STAT3 activation in tamoxifen-resistant breast cancer. Proc Natl Acad Sci USA. 2020 Jun 30;117(26):15047-54. doi: 10.1073/pnas.1910278117, PMID 32532922, PMCID PMC7334450.
52. Kim MS, Jeong J, Seo J, Kim HS, Kim SJ, Jin W. Author correction: dysregulated JAK2 expression by TrkC promotes metastasis potential, and EMT program of metastatic breast cancer. Sci RepSci Rep. 2020;10(1):11103. doi: 10.1038/s41598-020-68128-6, PMID 32632170.
53. Schneider J, Jeon YW, Suh YJ, Lim ST. Effects of ruxolitinib and calcitriol combination treatment on various molecular subtypes of breast cancer. Int J Mol Sci. 2022 Feb 25;23(5):2535. doi: 10.3390/ijms23052535, PMID 35269680, PMCID PMC8910493.
54. Gong Y, Zhao W, Jia Q, Dai J, Chen N, Chen Y. IKBKB rs2272736 is associated with gastric cancer survival. Pharmgenomics Pers Med. 2020 Aug 19;13:345-52. doi: 10.2147/PGPM.S258761, PMID 32884329, PMCID PMC7443400.
55. Pan F, Zhang J, Tang B, Jing L, Qiu B, Zha Z. The novel circ_0028171/miR-218-5p/IKBKB axis promotes osteosarcoma cancer progression. Cancer Cell Int. 2020 Oct 6;20:484. doi: 10.1186/s12935-020-01562-8, PMID 33041665, PMCID PMC7542388.
56. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L. Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018 May 12;5(2):77-106. doi: 10.1016/j.gendis.2018.05.001, PMID 30258937, PMCID PMC6147049.
57. Song D, Cui M, Zhao G, Fan Z, Nolan K, Yang Y. Pathway-based analysis of breast cancer. Am J Transl Res. 2014 May 15;6(3):302-11, PMID 24936222, PMCID PMC4058311.
58. Yousefnia S, Seyed Forootan F, Seyed Forootan S, Nasr Esfahani MH, Gure AO, Ghaedi K. Mechanistic pathways of malignancy in breast cancer stem cells. Front Oncol. 2020 Apr 30;10:452. doi: 10.3389/fonc.2020.00452, PMID 32426267, PMCID PMC7212408.